ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998276767 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9614583
TITLE: FasL induces Fas/Apo1-mediated **apoptosis** in human embryonic kidney 293 cells routinely used to generate E1-deleted **adenoviral** vectors.
AUTHOR: Larregina A T; Morelli A E; Dewey R A; Castro M G; Fontana A; Lowenstein P R
CORPORATE SOURCE: Department of Medicine, University of Manchester, UK.
SOURCE: Gene therapy, (1998 Apr) 5 (4) 563-8.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980708
Last Updated on STN: 19980708
Entered Medline: 19980622

AB Human embryonic kidney 293 cells contain the E1 region of **adenovirus** type 5, and thus sustain, through transcomplementation, the production of recombinant E1-deleted **adenovirus** vectors. During attempts to produce recombinant **adenovirus** expressing the **apoptosis**-inducing molecule Fas ligand (FasL) under the control of a very strong truncated major immediate-early human cytomegalovirus (MIEhCMV) promoter, we discovered that 293 cells were not surviving the initial cotransfection with a shuttle plasmid encoding the mouse FasL; and pJM17, a plasmid containing the genome of **adenovirus** type 5 with deletions in the E1-E3 regions, in an unpackagable form. Investigation of the reason for massive cell death after cotransfection led us to determine that 293 cells express the FasL receptor. Fas-Apo1 (CD95), and respond with **apoptosis** to the cross-linking of Fas-Apo1 with either IgM monoclonal antibodies or FasL. Therefore, we decided to generate **adenoviral** vectors expressing FasL, under the control of **tissue-specific** and/or-inducible **promoter** elements. Our findings can explain difficulties several groups have had in generating recombinant **adenoviral** vectors expressing FasL using 293 cells, as well as the lower titres reported.

L19 ANSWER 2 OF 2 MEDLINE on STN
ACCESSION NUMBER: 1998098362 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9436031
TITLE: Clinical application for gene therapy in prostate cancer.
AUTHOR: Gotoh A; Kamidono S; Chung L W
CORPORATE SOURCE: Department of Urology, Kobe University School of Medicine.
SOURCE: Hinyokika kiyo. Acta urologica Japonica, (1997 Nov) 43 (11) 829-33.
Journal code: 0421145. ISSN: 0018-1994.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980306
Last Updated on STN: 19980306
Entered Medline: 19980224

AB Hormone treatment, radiotherapy and anti-cancer chemotherapy are often used to treat prostate cancer. However, there is no effective method of treating hormone-independent prostate cancer. In this study, we attempted to establish a new treatment method for hormone-independent prostate cancer. We developed a new recombinant **adenovirus** vector containing a suicide gene and controlled by a **tissue specific promoter**, and examined the usefulness of gene

therapy for hormone-independence and PSA expression in prostate cancer. We have also examined the usefulness of gene therapy involving an **adenovirus** and various tumor suppressor genes for human prostate cancer cells, which are under trial in the United States.

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ACCESSION NUMBER: 2001418735 EMBASE
TITLE: A complex **adenovirus** vector that delivers FASL-GFP with combined prostate-specific and tetracycline-regulated expression.
AUTHOR: Rubinchik S.; Wang D.; Yu H.; Fan F.; Luo M.; Norris J.S.; Dong J.-Y.
CORPORATE SOURCE: J.-Y. Dong, Dept. of Microbiology and Immunology, Medical University of South Carolina, Charlestown, SC 29403, United States. dongj@musc.edu
SOURCE: Molecular Therapy, (2001) 4/5 (416-426).
Refs: 50
ISSN: 1525-0016 CODEN: MTOHCK
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
026 Immunology, Serology and Transplantation
028 Urology and Nephrology
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Cell-type-restricted transgene expression delivered by **adenovirus** vectors is highly desirable for gene therapy of cancer, as it can limit cytotoxic gene expression to tumor cells. However, many tumor- and **tissue-specific** promoters are weaker than the constitutively active promoters and are thus less effective. To combine cell-type specificity with high-level regulated transgene expression, we have developed a complex **adenoviral** vector. We have placed the **tetracycline** transactivator gene under the control of a prostate-specific ARR2PB **promoter**, and a mouse Tnfsf6 (encoding FASL)-GFP fusion gene under the control of the **tetracycline** responsive **promoter**. We have incorporated both expression cassettes into a single construct. We show that FASL-GFP expression from this vector is essentially restricted to prostate cancer cells, in which it can be regulated by **doxycycline**. Higher levels of prostate-specific FASL-GFP expression were generated by this approach than by driving the FASL-GFP expression directly with ARR2PB. More FASL-GFP expression correlated with greater induction of apoptosis in prostate cancer LNCaP cells. Mouse studies confirmed that systemic delivery of both the prostate-specific and the prostate-specific/tet-regulated vectors was well tolerated at doses that were lethal for FASL-GFP vector with CMV **promoter**. This strategy should be able to improve the safety and efficacy of cancer gene therapy using other cytotoxic genes as well.

L23 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:723195 CAPLUS
DOCUMENT NUMBER: 131:318578
TITLE: Partially deleted **adenoviral** vectors with therapeutic expression potential for transgenes where deleted vector genes are introduced within producer cell chromosome
INVENTOR(S): Wadsworth, Samuel C.; Scaria, Abraham
PATENT ASSIGNEE(S): Genzyme Corp., USA
SOURCE: PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9957296	A1	19991111	WO 1999-US9590	19990430
W: AU, CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2328087	AA	19991111	CA 1999-2328087	19990430
AU 9938770	A1	19991123	AU 1999-38770	19990430
EP 1075532	A1	20010214	EP 1999-921601	19990430
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002513582	T2	20020514	JP 2000-547249	19990430

PRIORITY APPLN. INFO.: US 1998-83841P P 19980501
US 1999-118118P P 19990201
WO 1999-US9590 W 19990430

AB The invention is directed to novel partially deleted **adenoviral** vectors (DeAd) in which the majority of **adenoviral** early genes required for replication are deleted from the vector and placed within the chromosome of a producer cell line under **conditional promoter** control. Rephrased, the expression of genes encoding virion structural proteins is made **conditional** by replacement of the major late **promoter** with alternative promoters that can be controlled.. Moreover, the procedures described here is directed to DeAd vectors in which expression of genes encoding virion structural proteins in diminished by deletion the VA RNA genes from the vector. This system is applicable to human **adenovirus** 2, 5, 6, and 17. The partially deleted **adenoviral** (DeAd) vectors of the invention can accommodate inserts, such as transgenes, of up to 12-15 kb in size. The invention is further directed to DeAd vector producer cell lines that contain the **adenoviral** early genes necessary for replication under **conditional promoter** control that allow for large scale production of vectors. This **conditional promoter** system includes control sequences from the dimerizer gene or **tetracycline** or ecdysone control systems. The invention is also directed to methods for the production of DeAd vectors in such cell lines and to the use of such vectors to deliver transgenes to target cells. These transgenes include the CFTR and human α-galactosidase A and erythropoietin and factor VII and factor IX.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 3 OF 4 EMBASE
on STN

ACCESSION NUMBER: 1999290209 EMBASE
TITLE: Tetracycline-mediated regulation of gene expression within the human cytomegalovirus genome.
AUTHOR: McVoy M.A.; Mocarskit E.S.
CORPORATE SOURCE: M.A. McVoy, Department of Pediatrics, Medical College of Virginia, Campus of Virginia Cmw. University, Richmond, VA 23298-0163, United States. mmcvoy@hsc.vcu.edu
SOURCE: Virology, (5 Jun 1999) 258/2 (295-303).
Refs: 39
ISSN: 0042-6822 CODEN: VIRLAX
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB To evaluate the utility of **tetracycline** gene regulation in the study of human cytomegalovirus gene functions, expression of luciferase under the control of **tetracycline**-regulatable promoters was studied following transient plasmid transfections and from within recombinant human cytomegalovirus genomes. The **tetracycline**

-regulatable **promoter** P(hCMV)(*-1) contains sequences from the human cytomegalovirus ie1/ie2 **promoter** and seven upstream tet operator sites which bind the activator protein tTA only in the absence of **tetracycline** (Gossen and Bujard (1992). Proc. Natl. Acad. Sci. USA 89, 5547-5551). Two modifications of P(hCMV)(*-1) were also studied: P1129, in which the tet operator sites were reduced from seven to one; and P1125, in which human cytomegalovirus sequences were replaced by **adenovirus** major late **promoter** and terminal deoxynucleotidyltransferase initiator sequences. In transient assays, P(hCMV)(*-1) and P1125 exhibited modest differential regulation but were strongly activated by viral infection. P1129 exhibited less viral activation and narrower regulation. In the viral genome, P(hCMV)(*-1 exhibited regulation up to 7-fold during late times of infection, whereas P1125 displayed nearly 100-fold regulation. Regulation of P1125 was fully reversed within 12 to 24 h of adding or removing **tetracycline**. These results suggest that P1125 may provide sufficient **conditional** expression to effectively regulate human cytomegalovirus late genes.

L23 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1999129183 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9930322
TITLE: Highly controlled gene expression using combinations of a **tissue-specific promoter**, recombinant **adenovirus** and a **tetracycline** -regulatable transcription factor.
AUTHOR: Ghersa P; Gobert R P; Sattonnet-Roche P; Richards C A; Merlo Pich E; Hooft van Huijsduijnen R
CORPORATE SOURCE: Serono Pharmaceutical Research Institute (previously Glaxo-Wellcome), Geneva, Switzerland.
SOURCE: Gene therapy, (1998 Sep) 5 (9) 1213-20. Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990311
Last Updated on STN: 19990311
Entered Medline: 19990225

AB Controllable gene expression is a desirable feature both in gene therapy protocols and for the study of gene function in animals and plants. We have exploited the modular character of the tetracycline (tc)-regulatable genetic switch to show that its components can be encoded by any combination of recombinant **adenovirus** and/or transgenic mice. Transgenic mice were constructed that express the tc-regulatable trans-activator tTA muscle specifically. These were injected with recombinant **adenovirus** expressing a luciferase reporter controlled by the tTA-regulatable promoter. Virus injected into muscle, but not into a control organ (brain) resulted in luciferase activity. Conversely, injection of tTA producing **adenovirus** into mice that were transgenic for a trkB/Fc fusion protein gene under tc promoter control resulted in swift expression of serum trkB/Fc receptor-body. Both modes of gene induction were fully inhibited by administration of tc. We demonstrate that a careful choice of these tools allows exquisite in vivo control over transgene expression in a temporal, tc-regulatable, topical and tissue-specific manner.

ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998290618 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9628654
TITLE: Development of prostate-specific antigen promoter-based gene therapy for androgen-independent human prostate cancer.
AUTHOR: Gotoh A; Ko S C; Shirakawa T; Cheon J; Kao C; Miyamoto T; Gardner T A; Ho L J; Cleutjens C B; Trapman J; Graham F L; Chung L W
CORPORATE SOURCE: Department of Urology, Molecular Urology and Therapeutics Program, University of Virginia, Charlottesville 22908, USA.
CONTRACT NUMBER: 1R29CA74042-01 (NCI)
SOURCE: Journal of urology, (1998 Jul) 160 (1) 220-9.
Journal code: 0376374. ISSN: 0022-5347.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980723
Last Updated on STN: 19980723
Entered Medline: 19980714

AB PURPOSE: The goal of this study is to develop a **tissue-specific** toxic gene therapy utilizing the prostate specific antigen (PSA) **promoter** for both **androgen**-dependent (AD) and **androgen**-independent (AI) PSA-secreting prostate cancer cells. Ideally this gene therapy would be effective without the necessity of exposing the target cells to circulating androgens. MATERIALS AND METHODS: An AI subline of LNCaP, an AD PSA-secreting human prostate cancer cell line, C4-2, was used in this study. Castrated mice bearing C4-2 tumors secrete PSA. A transient expression experiment was used to analyze the activity of two PSA promoters, a 5837 bp long PSA promoter and a 642 bp short PSA promoter, in C4-2 cells. A recombinant **adenovirus** (Ad-PSA-TK) carrying thymidine kinase under control of the long PSA promoter was generated. The tissue-specific activity of Ad-PSA-TK was tested in vitro and in vivo. RESULTS: The long PSA promoter had superior activity over short PSA promoter, and higher activity in C4-2 cells than in LNCaP cells. High activity of Ad-PSA-TK was observed in C4-2 cells in an **androgen** deprived condition. In vitro, Ad-PSA-TK was further demonstrated to induce marked C4-2 cell-kill by acyclovir in medium containing 5% FBS. No cell-kill was observed in control WH cells (a human bladder cancer cell line). In vivo, Ad-PSA-P-TK with acyclovir significantly inhibited subcutaneous C4-2 tumor growth and PSA production in castrated animals. CONCLUSION: The 5837 bp long PSA promoter was active in the **androgen** free environment and could be used to target both **androgen**-dependent and independent PSA-producing prostate cancer cells in vitro, and prostate tumors in castrated hosts.

L28 ANSWER 2 OF 4 EMBASE
on STN
ACCESSION NUMBER: 97046641 EMBASE
DOCUMENT NUMBER: 1997046641
TITLE: Regulation of androgen synthesis: The late steroidogenic pathway.
AUTHOR: Dufau M.L.; Miyagawa Y.; Takada S.; Khanum A.; Miyagawa H.; Buczko E.
CORPORATE SOURCE: M.L. Dufau, Endocrinol./Reproduc. Res. Branch, NICHHD, National Institutes of Health, Bethesda, MD, United States
SOURCE: Steroids, (1997) 62/1 (128-132).
Refs: 23
ISSN: 0039-128X CODEN: STEDAM
PUBLISHER IDENT.: S 0039-128X(96)00171-2

COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 003 Endocrinology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Studies of the regulation of **androgen** synthesis in steroidogenic cells have focused on both transcriptional and post-translational regulation of the proteins that catalyze these reactions: the P450c17 that catalyzes the production of DHEA or androstenedione in consecutive hydroxylase and lyase activities, and the 17β-hydroxysteroid dehydrogenase (17β-HSD) that catalyzes the conversion of androstenedione to testosterone. Our studies of the regulation of the CYP17 lyase activity at the molecular level have utilized species- and **tissue-specific** differences to identify target regulatory sequences. **Adenovirus** infection of rat CYP17 **promoter**/luciferase reporter gene constructs in primary cultures of rat adrenal and Rat Leydig cells revealed a rat-specific domain between -1 and -108 bp that cause inhibition of both basal and cAMP-induced CYP17 transcription in the adrenal, but not the Leydig cell. In contrast, similar **promoter** constructs from other species exhibited substantial cAMP-induced transcriptional activity in the rat adrenal. Mutagenesis of the conserved region of the rat and human proteins reveals significant differences in the amino acid domains required for hydroxylase and lyase activities within and between the two species, consistent with their differential regulation of lyase activity. The 17β-hydroxysteroid dehydrogenase (17β-HSD) reaction requires a viable glucose transporter system for optimal activity, and a high-energy phosphate was discovered to be the requisite product of glucose metabolism in 17β-HSD activation. These studies have provided insight into potential mechanisms of control of **androgen** synthesis in the late steroidogenic pathway, at the transcriptional and post-translational levels.

L28 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 92017873 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1922089
TITLE: Androgen modulation of DNA-binding factors in the mouse kidney.
AUTHOR: Rhee M; Dimaculangan D; Berger F G
CORPORATE SOURCE: Department of Biological Sciences, University of South Carolina, Columbia 29208.
CONTRACT NUMBER: DK-37265 (NIDDK)
SOURCE: Molecular endocrinology (Baltimore, Md.), (1991 Apr) 5 (4) 564-72.
Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199110
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911029

AB Transcription of the RP2 gene in the mouse kidney is induced by androgens. This induction is species specific within the genus Mus. For example, the gene responds to androgens in Mus domesticus, but is refractory to hormone in the distantly related species M. caroli. In the present report we have characterized DNA-binding factors that recognize the 5' flanking region of the RP2 gene. One factor (termed RPBF-1) binds a DNA fragment spanning the region between -157 and -311 relative to the transcriptional start site. RPBF-1 is present in kidney nuclear extracts from both control and **androgen**-treated M. domesticus as well as from control M. caroli;

however, in the latter species a distinct factor (termed RPBF-2) is induced by androgens and replaces RPBF-1. The **androgen**-dependent replacement of RPBF-1 by RPBF-2 is specific to the kidney of M. caroli. DNase-1 footprinting analyses indicate that the two factors recognize distinct, yet overlapping, regions of the RP2 promoter: RPBF-1 binds the region between -247 and -269, while RPBF-2 binds the region between -265 and -290. The RPBF-2-binding site contains a sequence that is homologous to that recognized by nuclear factor-1 (NF-1), suggesting that RPBF-2 is a NF-1-like factor. This is supported by competition experiments with synthetic oligonucleotides corresponding to the NF-1-binding site within the **adenovirus** origin of replication. Thus, androgens can modulate, in a species- and **tissue-specific** manner, DNA-binding factors that recognize **promoter** regions of genes.(ABSTRACT TRUNCATED AT 250 WORDS)

L28 ANSWER 4 OF 4 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 89061617 EMBASE
DOCUMENT NUMBER: 1989061617
TITLE: Developmentally regulated male-specific transfactor(s) enable in vitro transcription of a cloned α2(u)-globulin gene.
AUTHOR: Sarkar P.; Feigelson P.
CORPORATE SOURCE: Institute of Cancer Research, Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032, United States
SOURCE: Molecular Endocrinology, (1989) 3/2 (342-348).
ISSN: 0888-8809 CODEN: MOENEN
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 003 Endocrinology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Selected members of the rat α2(u)-globulin gene family are expressed in several tissues, manifesting characteristic developmental and endocrine transcriptional control. Studies are now underway to identify the responsible cis sequences and transacting factors. We recently reported that the cloned rat α2(u)-globulin 207 gene manifests **tissue-specific androgen**-dependent expression; it is expressed in livers of male, but not female, transgenic mice. In the present study a portion of this gene (-639 to +1395) is used as an in vitro template in the presence of nuclear extracts derived from hepatic nuclei of prepubescent and mature male and female rats. α-Amanitin-sensitive in vitro transcription of the α2(u) 207 gene by extracts derived from mature male rats is highly active and is at least 13 times as rapid as that with preparations from mature female or immature animals. In contrast, the rate of transcription of a control template, the **adenoviral** late **promoter**, is the same with all of these nuclear extracts. S1 nuclease analysis and the size of the transcript indicate that transcription is initiated in vitro at the same site as it is in vivo and that it continues to the 3' terminus of the α2(u)-globulin template. Thus, cis sequences are present in this gene fragment which are controlled by developmentally regulated male transacting factors, enabling selective transcription of this α2(u)-globulin gene. Mixing experiments indicate that this transcriptional control is positive.

ESSION NUMBER: 1998157983 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9488717
TITLE: Interaction of the **adenovirus** 14.7-kDa protein with FLICE inhibits Fas ligand-induced **apoptosis**.
AUTHOR: Chen P; Tian J; Kovesdi I; Bruder J T
CORPORATE SOURCE: GenVec, Inc., Rockville, Maryland 20852, USA.
SOURCE: Journal of biological chemistry, (1998 Mar 6) 273 (10) 5815-20.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 20000303
Entered Medline: 19980407

AB **Adenovirus** type 5 encodes a 14.7-kDa protein that protects infected cells from **tumor** necrosis factor-induced cytolysis by an unknown mechanism. In this report, we demonstrate that infection of cells with an **adenovirus** vector expressing Fas ligand induced rapid **apoptosis** that was blocked by coinfection with a virus expressing 14. 7K. Moreover, AdFasL/G infection resulted in the rapid activation of DEVD-specific caspases, and caspase activation was blocked by coinfection with Ad14.7/G. Cell death induced by the overexpression of Fas ligand, Fas-associated death domain-containing protein (FADD)/MORT1, or FADD-like interleukin-1beta-converting enzyme (FLICE)/caspase-8 in a virus-free system was efficiently blocked by 14.7K expression. Moreover, we demonstrate that 14.7K interacts with FLICE. These results support the idea that FLICE is a cellular target for the 14.7-kDa protein.

L38 ANSWER 2 OF 20 MEDLINE on STN
ACCESSION NUMBER: 1999068406 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9853517
TITLE: Generation of fiber-mutant recombinant **adenoviruses** for gene therapy of malignant glioma.
AUTHOR: Yoshida Y; Sadata A; Zhang W; Saito K; Shinoura N; Hamada H
CORPORATE SOURCE: Department of Molecular Biotherapy Research, Cancer Chemotherapy Center, Cancer Institute, Tokyo, Japan.
SOURCE: Human gene therapy, (1998 Nov 20) 9 (17) 2503-15.
Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990316
Last Updated on STN: 19990316
Entered Medline: 19990226

AB Recombinant **adenovirus** (Adv)-mediated gene transduction is a powerful technology for **cancer** gene therapy. In this article, we report the generation of a fiber-mutant Adv vector, using the Adv genomic DNA-terminal protein complex (DNA-TPC) cotransfection method. First, a fiber-mutant construct in a plasmid carrying the right-side two-thirds of the human **adenovirus** type 5 (Ad5) genome (pTR) was cotransfected with Ad5 DNA-TPC, yielding the recombinant Adv with the desired fiber mutation. The DNA-TPC from the mutant Adv was then utilized to produce a second-step recombinant Adv with an expression cassette in the place of E1. By this procedure, we generated a fiber mutant, F/K20, that has a linker and a stretch of 20 lysine residues added at the C terminus of the fiber. By using Adv carrying a **reporter** lacZ gene (AxCAZ2) with either F/K20 or wild-type fiber (F/wt), we examined the transduction efficiency of F/K20-Adv. No significant difference in the

transduction efficiency between F/K20 and F/wt-Adv was observed for a human fibroblast line, WI-38, or various **tumor** cell lines, including melanoma, prostate, esophageal, and pancreatic **cancer** lines. In clear contrast, F/K20-Adv showed a remarkably enhanced efficiency in genetic transduction of human glioma cells. In all four human glioma lines tested, the multiplicities of infection (MOIs) for transduction of 50% of the population (ED50) were decreased with F/K20-Adv compared with F/wt-Adv: 7-fold for T98G, 14-fold for U251, 9-fold for U373, and 42-fold for U87 cells. Therefore, we attempted to apply F/K20-Adv for gene therapy of malignant glioma. Glioma cells infected with F/K20-Adv carrying genes for interleukin 2 or interleukin 12 produced a high level of each cytokine at a much lower MOI than did cells infected with F/wt-Adv. Infection with F/K20-Adv carrying the wild-type p53 **tumor** suppressor gene resulted in an enhanced level of p53 protein expression and an increased incidence of F/K20-Adv in transduction efficiency for malignant glioma, providing promising tools for gene therapy.

L38 ANSWER 3 OF 20 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998430734 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9759934
TITLE: A phase I study of **adenovirus**-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung **cancer**.
AUTHOR: Schuler M; Rochlitz C; Horowitz J A; Schlegel J; Perruchoud A P; Kommoss F; Bolliger C T; Kauczor H U; Dalquen P; Fritz M A; Swanson S; Herrmann R; Huber C
CORPORATE SOURCE: Department of Medicine III, Johannes Gutenberg University, Mainz, Germany.
SOURCE: Human gene therapy, (1998 Sep 20) 9 (14) 2075-82. Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: (CLINICAL TRIAL)
(CLINICAL TRIAL, PHASE I)
Journal; Article; (JOURNAL ARTICLE)
(MULTICENTER STUDY)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981211

AB Mutations of the **tumor** suppressor gene p53 are the most common genetic alterations observed in human **cancer**. Loss of wild-type p53 function impairs cell cycle arrest as well as repair mechanisms involved in response to DNA damage. Further, apoptotic pathways as induced by radio- or chemotherapy are also abrogated. Gene transfer of wild-type p53 was shown to reverse these deficiencies and to induce **apoptosis** in vitro and in preclinical in vivo **tumor** models. A phase I dose escalation study of a single intratumoral injection of a replication-defective **adenoviral** expression vector encoding wild-type p53 was carried out in patients with incurable non-small cell lung **cancer**. All patients enrolled had p53 protein overexpression as a **marker** of mutant p53 status in pretreatment **tumor** biopsies. Treatment was performed either by bronchoscopic intratumoral injection or by CT-guided percutaneous intratumoral injection of the vector solution. Fifteen patients were enrolled in two centers, and were treated at four different dose levels ranging from 10(7) to 10(10) PFU (7.5 x 10(9) to 7.5 x 10(12) particles). No clinically significant toxicity was observed. Successful transfer of wild-type p53 was achieved only with higher vector doses. Vector-specific wild-type p53 RNA sequences could be demonstrated in posttreatment biopsies of six patients. Transient local disease control by a single

intratumoral injection of the vector solution was observed in four of those six successfully transduced patients. There was no evidence of clinical responses at untreated **tumor** sites. Wild-type p53 gene therapy by intratumoral injection of a replication-defective **adenoviral** expression vector is safe, feasible, and biologically effective in patients with advanced non-small cell lung **cancer**.

L38 ANSWER 4 OF 20 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998240970 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9581814
TITLE: Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations.
AUTHOR: Tada M; Matsumoto R; Iggo R D; Onimaru R; Shirato H; Sawamura Y; Shinohe Y
CORPORATE SOURCE: Laboratory for Molecular Brain Research, Hokkaido University School of Medicine, Sapporo, Japan.. m_tada@med.hokudai.ac.jp
SOURCE: Cancer research, (1998 May 1) 58 (9) 1793-7. Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980611
Last Updated on STN: 20000303
Entered Medline: 19980602

AB Recent studies suggest that a balance may exist between the cell cycle arrest and **apoptosis**-inducing functions of the p53 **tumor** suppressor gene. **Adenoviral** p21 transduction attenuates **apoptosis**, whereas deletion of the p21 gene promotes it, and p21-null xenografts respond better than isogenic p21-wild type tumors to irradiation. Hence, the role of p53 in dictating the clinical response to radiotherapy and chemotherapy may be more complex than previously thought. We have analyzed survival and radiation response (regrowth-free period) of 42 patients with glioblastomas whose p53 status was determined by a sensitive yeast functional assay. Multivariate analysis revealed that p53 mutation is associated with longer survival ($P < 0.02$). Among 36 radiation-treated patients, the regrowth-free period after treatment was significantly longer for tumors with p53 mutations ($P < 0.0001$), and p53 mutation was the sole independent factor predictive of radiotherapeutic response ($P < 0.01$). Survival time after regrowth was independent of p53 status, suggesting that the difference in survival was related to the treatment rather than to the intrinsic aggressiveness of the **tumor** . Thus, in this Northern Japanese population, p53 mutation is a **marker** for better radiation response in glioblastomas, and this results in significantly longer survival.

L38 ANSWER 5 OF 20 MEDLINE on STN
ACCESSION NUMBER: 1999029693 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9814556
TITLE: Overexpression of glia maturation factor in C6 cells promotes differentiation and activates superoxide dismutase.
AUTHOR: Lim R; Zaheer A; Kraakevik J A; Darby C J; Oberley L W
CORPORATE SOURCE: Department of Neurology, University of Iowa College of Medicine and Veterans Affairs Medical Center, Iowa City 52242, USA.
CONTRACT NUMBER: DK-25295 (NIDDK)
P01-CA66081 (NCI)
P50 DE-10758 (NIDCR)
SOURCE: Neurochemical research, (1998 Nov) 23 (11) 1445-51. Journal code: 7613461. ISSN: 0364-3190.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990308

AB In order to evaluate the intracellular function of glia maturation factor (GMF), we overexpressed GMF in C6 rat glioma cells using two methods: stable transfection using the pcDNA3 plasmid, and transient transfection using replication-defective human **adenovirus**. With both methods, C6 cells transfected with GMF and overexpressing the protein exhibit a lower saturation density in culture compared to non-transfected or vector alone controls. Transfected cells also exhibit morphological differentiation as shown by the outgrowth of cell processes. When inoculated into nude mice, transfected cells are less tumorigenic than controls, and express the mature astrocytic **marker** glial fibrillary acidic protein. In tissue culture, transfected cells show a 3.5-fold increase in CuZn-dependent superoxide dismutase (CuZnSOD) activity. Western blot analysis reveals a 3.5-fold increase in CuZnSOD protein, suggesting an induction of the enzyme. In view of recent findings that reactive oxygen species (ROS) and the antioxidant enzymes are intricately involved in key physiologic processes such as proliferation, differentiation and **apoptosis**, the study raises the possibility that CuZnSOD may be a mediator of GMF function.

L38 ANSWER 6 OF 20 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1998451286 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9780006
TITLE: Reduced telomeric signals and increased telomeric associations in human lung **cancer** cell lines undergoing p53-mediated **apoptosis**.
AUTHOR: Mukhopadhyay T; Multani A S; Roth J A; Pathak S
CORPORATE SOURCE: Department of Thoracic and Cardiovascular Surgery, The University of Texas MD Anderson Cancer Center, Houston 77030, USA.
CONTRACT NUMBER: CA 16672 (NCI)
P50-CA70907 (NCI)
RO1 CA45187 (NCI)
SOURCE: Oncogene, (1998 Aug 20) 17 (7) 901-6.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 20020125
Entered Medline: 19981123

AB Transduction of a p53-negative H1299 human non-small cell lung **cancer** cell line with an **adenoviral** vector containing wild-type p53 (Ad5p53) induced **apoptosis**. Analysis of the Ad5p53-infected H1299 cells showed high levels of telomeric association prior to apoptotic nuclear fragmentation. Similar telomeric association was observed in stably transfected clones of the wtH226b cell line, which expressed exogenous wild-type p53 protein and also showed complex chromosomal abnormalities including dicentrics, rings and fragments. Fluorescence in situ hybridization (FISH) analysis using a human telomeric DNA probe indicated reductions in telomere signals in both Ad5p53-infected H1299 cells and wtH226b-S cells. In contrast, stably transfected wtH226b-AS clones expressing antisense p53 cDNA showed no telomeric association and had high levels of telomeric signals associated with a

faster growing phenotype. These results suggest that wild-type p53 is involved in shortening telomeres, a possibly early event in the p53-mediated apoptotic process and in the subsequent telomeric association that predisposes a cell to genetic instability and DNA fragmentation resulting in apoptotic cell death. Moreover, loss of telomeric signals may indicate a cell's decision to undergo programmed cell death and, if so, could, serve as a sensitive **marker** of p53-mediated **apoptosis**.

L38 ANSWER 7 OF 20 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999084858 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9869513
TITLE: **Adenovirus**-mediated wild-type p53 **tumor** suppressor gene therapy induces **apoptosis** and suppresses growth of human pancreatic **cancer** [seecomments].
COMMENT: Comment in: Ann Surg Oncol. 1998 Dec;5(8):667-9. PubMed ID: 9869510
AUTHOR: Bouvet M; Bold R J; Lee J; Evans D B; Abbruzzese J L; Chiao P J; McConkey D J; Chandra J; Chada S; Fang B; Roth J A
CORPORATE SOURCE: Department of Surgical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston 77030, USA.
CONTRACT NUMBER: CA 16672 (NCI)
T32-09599-08
SOURCE: Annals of surgical oncology : official journal of the Society of Surgical Oncology, (1998 Dec) 5 (8) 681-8. Journal code: 9420840. ISSN: 1068-9265.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20020912
Entered Medline: 19990308

AB BACKGROUND: The p53 **tumor** suppressor gene is mutated in up to 70% of pancreatic adenocarcinomas. We determined the effect of reintroduction of the wild-type p53 gene on proliferation and **apoptosis** in human pancreatic **cancer** cells using an **adenoviral** vector containing the wild-type p53 **tumor** suppressor gene. METHODS: Transduction efficiencies of six p53-mutant pancreatic **cancer** cell lines (AsPC-1, BxPC-3, Capan-1, CFPAC-1, MIA PaCa-2, and PANC-1) were determined using the **reporter** gene construct Ad5/CMV/beta-gal. Cell proliferation was monitored using a 3H-thymidine incorporation assay, Western blot analysis for p53 expression was performed, and DNA laddering and fluorescence-activated cell sorter analysis were used to assess **apoptosis**. p53 gene therapy was tested in vivo in a subcutaneous **tumor** model. RESULTS: The cell lines varied in transduction efficiency. The MIA PaCa-2 cells had the highest transduction efficiency, with 65% of pancreatic **tumor** cells staining positive for beta-galactosidase (beta-gal) at a multiplicity of infection (MOI) of 50. At the same MOI, only 15% of the CFPAC-1 cells expressed the beta-gal gene. **Adenovirus**-mediated p53 gene transfer suppressed growth of all human pancreatic **cancer** cell lines in a dose-dependent manner. Western blot analysis confirmed the presence of the p53 protein product at 48 hours after infection. DNA ladders demonstrated increased chromatin degradation, and fluorescence-activated cell sorter analysis demonstrated a four-fold increase in apoptotic cells at 48 and 72 hours following infection with Ad5/CMV/p53 in the MIA PaCa-2 and PANC-1 cells. Suppression of **tumor** growth mediated by induction of **apoptosis** was observed in vivo in an established nude mouse subcutaneous **tumor** model following intratumoral injections of Ad5/CMV/p53. CONCLUSIONS:

Introduction of the wild-type p53 gene using an **adenoviral** vector in pancreatic **cancer** with p53 mutations induces **apoptosis** and inhibits cell growth. These data provide preliminary support for **adenoviral** mediated p53 **tumor** suppressor gene therapy of human pancreatic **cancer**.

L38 ANSWER 8 OF 20 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999014598 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9797864
TITLE: Efficacy of multiple administrations of a recombinant **adenovirus** expressing wild-type p53 in an immune-competent mouse **tumor** model.
AUTHOR: Li Z; Rakkar A; Katayose Y; Kim M; Shanmugam N; Srivastava S; Moul J W; McLeod D G; Cowan K H; Seth P
CORPORATE SOURCE: Medical Breast Cancer Section, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.
SOURCE: Gene therapy, (1998 May) 5 (5) 605-13.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990128
Last Updated on STN: 19990128
Entered Medline: 19990113

AB Infection of Renca cells in vitro with a recombinant **adenovirus** expressing a **marker** gene beta-galactosidase resulted in high level of the transgene expression. Renca tumors grown in Balb/C mice were also infectable with this recombinant **adenovirus**. The transgene expression in the tumors lasted for about 7 days, however, administration of another dose of Ad-beta gal, on day 7 produced beta-galactosidase expression. To investigate the effect of antibodies to **adenovirus** , animals were injected with multiple doses of **adenovirus** to produce neutralizing antibodies. To these animals Renca cells were injected and tumors formed. Interestingly, when Ad beta-gal was administered into these tumors, a high level of transgene expression was still observed. We next explored the utility of a recombinant **adenovirus** expressing p53 (AdWTp53) in the Renca **tumor** model. Renca cells when exposed to an **adenovirus** expressing p53 (AdWTp53) produced a high level of p53 protein, a p53-inducible gene p21/WAF1/Cip1 and underwent **apoptosis**. A single injection of AdWTp53 (10(9) plaque forming units) resulted in significant inhibition of **tumor** growth. However, multiple administrations (four doses of 2.5 x 10(8) plaque forming units) of AdWTp53 were needed for **tumor** cures. Mixing uninfected and AdWTp53-infected cells showed a bystander effect of AdWTp53-infected Renca cells. Based on these results we believe that an appropriate dose scheduling of AdWTp53 can be efficacious for **cancer** gene therapy in immune-competent **tumor**-bearing animals.

L38 ANSWER 9 OF 20 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1998393418 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9726817
TITLE: RRR-alpha-tocopheryl succinate induction of prolonged activation of c-jun amino-terminal kinase and c-jun during induction of **apoptosis** in human MDA-MB-435 breast **cancer** cells.
AUTHOR: Yu W; Simmons-Menchaca M; You H; Brown P; Birrer M J; Sanders B G; Kline K
CORPORATE SOURCE: Department of Zoology, The University of Texas at Austin, 78712-1097, USA.
CONTRACT NUMBER: CA 59739 (NCI)

SOURCE: Molecular carcinogenesis, (1998 Aug) 22 (4) 247-57.
Journal code: 8811105. ISSN: 0899-1987.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 19980925
Entered Medline: 19980915

AB We have demonstrated that RRR-alpha-tocopheryl succinate (10 microg/mL vitamin E succinate (VES) treatment of estrogen receptor-negative MDA-MB-435 human breast **cancer** cells induces 9, 19, 51, and 72% apoptotic cells on days 1-4, respectively, after treatment, which involves transforming growth factor-beta signaling. Here, we show that VES-triggered **apoptosis** of MDA-MB-435 cells induced prolonged elevated expression of c-jun mRNA and protein (neither of which was caused by major increases in stability) and also induced enhanced activator protein-1 (AP-1) binding to the consensus DNA oligomer. Furthermore, VES treatments resulted in increased AP-1 transactivation activity, as measured with an AP-1 promoter/luciferase **reporter** construct and by the measurement of increased mRNA expression of the AP-1-dependent endogenous gene collagenase. Evidence of VES-induced involvement of the c-jun amino-terminal kinase in these AP-1-dependent events was suggested by data showing prolonged activity of this kinase, as measured by a kinase assay using glutathione S-transferase-c-jun as the substrate. The c-jun-dependent transcriptional activity was verified by cotransfection of a chimeric transcription factor having a galactose 4 DNA-binding domain coupled with the transactivation domain of c-jun plus the **reporter** plasmid 5X GAL4-luciferase. MDA-MB-435 cells infected with an **adenovirus** expression vector containing the TAM-67 sequence for dominant/negative-acting mutant c-jun or transiently transfected with c-jun antisense exhibited a 50-77% reduction in VES-mediated **apoptosis** as compared with control **adenovirus**-infected or control sense oligomer-transfected cells.

L38 ANSWER 10 OF 20 EMBASE
on STN
ACCESSION NUMBER: 1998231305 EMBASE
TITLE: Studies on the molecular mechanism of growth inhibition with p53 **adenoviral** construct in human ovarian **cancer**.
AUTHOR: Mujoo K.; Catino J.J.; Maneval D.C.; Gutterman J.U.
CORPORATE SOURCE: Dr. K. Mujoo, Department of Molecular Oncology, Box 41, Texas M.D. Univ. Anderson Can. Ctr., 1515 Holcombe, Houston, TX 77030, United States
SOURCE: International Journal of Gynecological Cancer, (1998) 8/3 (233-241).
Refs: 28
ISSN: 1048-891X CODEN: IJGCEN
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 010 Obstetrics and Gynecology
016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Advanced stage human ovarian **cancer** exhibits 50-60% mutation of the p53 **tumor** suppressor gene. We introduced the wild-type p53 gene into the cells using a replication deficient recombinant **adenovirus** for p53 gene therapy. p53-**adenovirus** (rAd-p53) inhibited the growth of a number of ovarian **cancer** cells, which correlated well with the transduction of **adenovirus** containing β-galactosidase **reporter** gene in the tested cell

lines. Results presented herein demonstrate that p53 induced the expression of CDK inhibitor WAF1/CIP1/p21 in human ovarian **cancer** cells with null or mutant p53. p53 incorporation also induced the expression of mdm-2 and bax proteins in human ovarian **cancer** cells. In contrast, we were unable to detect the expression of bcl-2 protein in the tested cells, and the expression bcl-x(L) in the tested human ovarian cells was not altered post-infection of cells with rAd-p53. Cell cycle analysis revealed pronounced G1 arrest 24 h post-infection with rAd-p53 in human ovarian **cancer** cells with only a small percentage of cells (-2%) undergoing **apoptosis**. rAd-p53 (p53-**adenovirus**) inhibited the growth of established subcutaneous xenograft tumors (OVCAR-3) of human ovarian carcinoma and completely regressed the tumors in 5/8 mice, indicating a potential for p53 **tumor** suppressor gene therapy in human ovarian **cancer**.

L38 ANSWER 11 OF 20 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1998312619 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9650599
TITLE: Transfection of a vector expressing wild-type p53 into cells of two human glioma cell lines enhances radiation toxicity.
AUTHOR: Geng L; Walter S; Melian E; Vaughan A T
CORPORATE SOURCE: Loyola-Hines Department of Radiotherapy, Cancer Center #338, Maywood, Illinois 60153, USA.
SOURCE: Radiation research, (1998 Jul) 150 (1) 31-7. Journal code: 0401245. ISSN: 0033-7587.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980723
Last Updated on STN: 19980723
Entered Medline: 19980716

AB Replication-deficient **adenovirus** (Adv5)-based vectors containing either wild-type p53 or the beta-gal **marker** gene were introduced into cells of the T98G (p53 mutant) and U87MG (p53 wild-type) human glioma cell lines. The wild-type p53 gene was successfully expressed in each cell line as shown by flow cytometry and Western blotting. The presence of the p53-expressing vector was toxic in both cell lines compared to control cells or to those containing the beta-gal vector. At levels of Adv5p53 vector that produced detectable toxicity, the effect of irradiation was enhanced, producing a twofold increase in cell killing. In the T98G cells, the presence of the p53 vector resulted in an increase in the number of cells undergoing **apoptosis** after irradiation, whereas a smaller and only additive response was observed in the U87MG cells. Conversely, an increase in micronucleus formation, indicating corrupt mitotic activity, was observed in irradiated Adv5p53-positive U87MG cells but not in T98G cells. These data suggest that p53-expressing vectors effectively enhance radiation lethality in these human glioma cell lines, but that the mechanism of action cannot be simply related to activation of the p53-dependent pathway to **apoptosis**.

L38 ANSWER 12 OF 20 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 97319614 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9176517
TITLE: An intracellular anti-erbB-2 single-chain antibody is specifically cytotoxic to human breast carcinoma cells overexpressing erbB-2.
AUTHOR: Wright M; Grim J; Deshane J; Kim M; Strong T V; Siegal G P; Curiel D T
CORPORATE SOURCE: Gene Therapy Program, University of Alabama at Birmingham 35294, USA.

CONTRACT NUMBER: CA 69343-01 (NCI)
SOURCE: Gene therapy, (1997 Apr) 4 (4) 317-22.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970709
Last Updated on STN: 20000303
Entered Medline: 19970623

AB We previously demonstrated that delivery of a gene encoding an anti-erbB-2 intracellular single-chain antibody (sFv) resulted in down-regulation of cell surface erbB-2 levels and induction of **apoptosis** in erbB-2 overexpressing ovarian **cancer** cells. Based upon these findings, we hypothesized that human breast carcinomas overexpressing erbB-2 would be similarly affected by this genetic intervention. We evaluated the phenotypic effects resulting from intracellular expression of the anti-erbB-2 sFv on the human breast **cancer** cell lines MDA-MB-361, SK-BR-3, BT-474, MCF-7 and MDA-MB-231. Recombinant **adenoviruses** encoding either a **reporter** gene (AdCMVLacZ) or the endoplasmic reticulum (ER) directed anti-erbB-2 sFv (Ad21) were delivered to various breast **cancer** cell lines. Cell viability was determined by a proliferation assay and fluorescent microscopy allowed visualization of apoptotic cells. An erbB-2 ELISA quantified the endogenous erbB-2 levels of each cell line. The anti-erbB-2 sFv-encoding-**adenovirus**, Ad21, but not the beta-galactosidase encoding **adenovirus**, AdCMVLacZ, was cytotoxic to > 95% of the **tumor** cells in the MDA-MB-361 and SK-BR-3 lines, and > 60% of the **tumor** cells in the BT-474 line. In marked contrast, the MCF-7 and MDA-MB-231 cell lines showed no change in the rate of cell proliferation following this treatment. The cytotoxic effects generated in the first three lines were a consequence of the induction of **apoptosis** by the anti-erbB-2 sFv. An ELISA specific for erbB-2 showed that the breast **cancer** cell lines most susceptible to the anti-erbB-2 sFv, MDA-MB-361, SK-BR-3 and BT-474, overexpressed the erbB-2 protein while the cell lines demonstrating no response to the anti-erbB-2 sFv, MCF-7 and MDA-MB-231, expressed the lowest levels of erbB-2. These results demonstrate that targeted killing of erbB-2 overexpressing cells via intracellular knockout can be accomplished in the context of breast carcinoma. Furthermore, erbB-2 levels in breast **tumor** cells may be predictive of their sensitivity to sFv-mediated killing. The ability to accomplish selective cytotoxicity of breast **cancer** cell lines overexpressing the erbB-2 **tumor marker** should allow for derivation of clinical gene therapy strategies for breast **cancer** utilizing this approach.

L38 ANSWER 13 OF 20 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:230256 BIOSIS
DOCUMENT NUMBER: PREV199799529459
TITLE: Growth inhibition and **apoptosis** of human ovarian **cancer** with p53-**adenoviral** construct.
AUTHOR(S): Mujoo, K.; Catino, J.; Maneval, D.; Gutterman, J.
CORPORATE SOURCE: M.D. Anderson Cancer Cent., Houston, TX 77030, USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (1997) Vol. 38, No. 0, pp. 8.
Meeting Info.: Eighty-eighth Annual Meeting of the American Association for Cancer Research. San Diego, California, USA. April 12-16, 1997.
ISSN: 0197-016X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English

ENTRY DATE: Entered STN: 2 Jun 1997
Last Updated on STN: 2 Jun 1997

L38 ANSWER 14 OF 20 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1999035193 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9816113
TITLE: Evaluation of topical gene therapy for head and neck squamous cell carcinoma in an organotypic model.
AUTHOR: Eicher S A; Clayman G L; Liu T J; Shillitoe E J; Storthz K A; Roth J A; Lotan R
CORPORATE SOURCE: Departments of Head and Neck Surgery, Thoracic and Cardiovascular Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA.
CONTRACT NUMBER: CA-16672 (NCI)
SOURCE: Clinical cancer research : an official journal of the American Association for Cancer Research, (1996 Oct) 2 (10) 1659-64.
Journal code: 9502500. ISSN: 1078-0432.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990311
Last Updated on STN: 19990311
Entered Medline: 19990225

AB The organotypic (raft) culture system has been shown to be a useful model for examining the effects of biochemical manipulations on various epithelial cell types, using in vitro conditions that simulate the in vivo environment of the tissue of origin. To investigate this method as a model for topical gene therapy, we cultured the oral head and neck squamous cell carcinoma cell line TR146 on fibroblast-containing collagen gels at the air-medium interface and assessed the efficiency of transduction of a topically applied **adenoviral** vector containing beta-galactosidase cDNA. Diffuse expression of -galactosidase activity in multiple cell layers demonstrated effective penetration of the vector. Transduction efficiency and therapeutic activity of a replication-defective recombinant **adenovirus** containing wild-type p53 cDNA linked to a FLAG **marker** (AdCMV-p53-FLAG) were then assessed in TR146 organotypic cultures transduced by topical application. Twenty-four, 48, and 72 h after transduction, the cultures were harvested, and residual cell number and FLAG peptide expression were determined. The number of cells in p53 transduced cultures was significantly reduced in comparison to controls at all three time points ($P < 0.001$), which resulted from the induction of **apoptosis** as determined by in situ DNA end labeling. In addition, the FLAG peptide was expressed diffusely in the residual cells, further confirming effective transduction and expression of the exogenous gene products throughout multiple layers. We conclude that the organotypic culture is an effective in vitro model for assessing the efficacy of topically applied gene therapy on head and neck squamous carcinomas and premalignancies.

L38 ANSWER 15 OF 20 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 97068009 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8911337
TITLE: Gene therapy for lung **cancer**: enhancement of **tumor** suppression by a combination of sequential systemic cisplatin and **adenovirus**-mediated p53 gene transfer.
AUTHOR: Nguyen D M; Spitz F R; Yen N; Cristiano R J; Roth J A
CORPORATE SOURCE: Department of Thoracic Surgery, University of Texas M.D. Anderson Cancer Center, Houston 77030, USA.
CONTRACT NUMBER: CA16672 (NCI)

R01 CA45187 (NCI)
R29 CA66037 (NCI)

SOURCE: Journal of thoracic and cardiovascular surgery, (1996 Nov) 112 (5) 1372-6; discussion 1376-7.
Journal code: 0376343. ISSN: 0022-5223.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961210

AB A more effective gene therapy strategy for lung **cancer** using sequential cisplatin administration and **adenovirus**-mediated p53 gene transfer was developed on the basis of our previous observation of enhanced expression of a **reporter** gene in malignant cells exposed to cisplatin before gene transfer. Transfer of the normal (wildtype) p53 gene into cisplatin-treated H1299 cells, in which p53 is homozygously deleted, resulted in up to a 60% further inhibition of cell proliferation in vitro than p53 transfer into untreated H1299 cells. The cisplatin plus p53 gene transfer strategy yielded significantly greater **apoptosis** and **tumor** growth suppression in an animal model of subcutaneous H1299 **tumor** nodules than wildtype p53 gene transfer alone. The timing of cisplatin administration and p53 gene transfer was shown to be critical: cisplatin administration simultaneous with or subsequent to p53 gene transfer was less effective than cisplatin-first sequential treatment. Moreover, the in vivo inhibition of **tumor** growth was maintained by repeated cycles of treatment. This gene therapy strategy has been incorporated into a phase I clinical trial for the treatment of lung **cancer** and provides a basis for the development of improved therapeutic protocols.

L38 ANSWER 16 OF 20 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 97081106 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8922388
TITLE: **Adenovirus**-mediated gene transfer of the **tumor** suppressor, p53, induces **apoptosis** in postmitotic neurons.
AUTHOR: Slack R S; Belliveau D J; Rosenberg M; Atwal J; Lochmuller H; Aloyz R; Haghighi A; Lach B; Seth P; Cooper E; Miller F D
CORPORATE SOURCE: Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, Canada.
SOURCE: Journal of cell biology, (1996 Nov) 135 (4) 1085-96.
Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970109

AB Programmed cell death is an ongoing process in both the developing and the mature nervous system. The **tumor** suppressor gene, p53, can induce **apoptosis** in a number of different cell types. Recently, the enhanced expression of p53 has been observed during acute neurological disease. To determine whether p53 overexpression could influence neuronal survival, we used a recombinant **adenovirus** vector carrying wild type p53 to transduce postmitotic neurons. A control consisting of the same **adenovirus** vector background but carrying the lacZ **reporter** expression cassette was used to establish working

parameters for the effective genetic manipulation of sympathetic neurons. We have found that recombinant **adenovirus** can be used at titers sufficiently high (10 to 50 multiplicity of infection) to transduce the majority of the neuronal population without perturbing survival, electrophysiological function, or cytoarchitecture. Moreover, we demonstrate that overexpression of wild type p53 is sufficient to induce programmed cell death in neurons. The observation that p53 is capable of inducing **apoptosis** in postmitotic neurons has major implications for the mechanisms of cell death in the traumatized mature nervous system.

L38 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

US 5,837,531

ACCESSION NUMBER: 1995:761795 CAPLUS
DOCUMENT NUMBER: 123:132861
TITLE: **Adenovirus** expression vectors using **tumor**-inducible expression cassettes for gene therapy in cancers
INVENTOR(S): Dedieu, Jean-Francois; Le, Roux Aude; Perricaudet, Michel
PATENT ASSIGNEE(S): Rhone-Poulenc Rorer S.A., Fr.
SOURCE: PCT Int. Appl., 24 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9514101	A1	19950526	WO 1994-FR1284	19941107
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
FR 2712602	A1	19950524	FR 1993-13766	19931118
FR 2712602	B1	19960209		
CA 2176585	AA	19950526	CA 1994-2176585	19941107
AU 9481471	A1	19950606	AU 1994-81471	19941107
AU 699867	B2	19981217		
EP 729516	A1	19960904	EP 1995-900795	19941107
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
JP 09504955	T2	19970520	JP 1994-514247	19941107
ZA 9409103	A	19950721	ZA 1994-9103	19941116
US 5837531	A	19981117	US 1996-646246	19960513
NO 9601977	A	19960514	NO 1996-1977	19960514
FI 9602114	A	19960517	FI 1996-2114	19960517

PRIORITY APPLN. INFO.: FR 1993-13766 A 19931118
WO 1994-FR1284 W 19941107

AB Viral expression vectors with a therapeutic gene under the control of expression signals specifically active in **tumor** cells, and their preparation and use in the treatment and prevention of cancers are described. The preferred virus is a replication-defective **adenovirus**. The gene may be a **tumor** suppressor gene, or it may encode a cytotoxin, a lymphokine, or a prodrug activating enzyme (such as a thymidine kinase). The promoter may be derived from an oncogenic virus. The construction of such vectors using a chimeric promoter derived from the Epstein-Barr nuclear antigen 1 and terminal protein 1 genes is demonstrated. EBNA1-dependent induction of **reporter** gene expression was demonstrated.

L38 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:762542 CAPLUS

DOCUMENT NUMBER: 123:277921
TITLE: p53 Stimulates transcription from the human transforming growth factor α promoter: a potential growth-stimulatory role for p53
AUTHOR(S): Shin, Tae Ho; Paterson, Andrew J.; Kudlow, Jeffrey E.
CORPORATE SOURCE: Departments Med. Cell Biol., Univ. Alabama Birmingham, Birmingham, AL, 35294, USA
SOURCE: Molecular and Cellular Biology (1995), 15(9), 4694-701
CODEN: MCEBD4; ISSN: 0270-7306
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Phys. and chemical agents can damage the genome. Part of the protective response to this damage is the increased expression of p53. P53, a transcription factor, controls the expression of genes, leading to cell cycle arrest and **apoptosis**. Another protective mechanism is the proliferative response required to replace the damaged cells. This proliferation is likely to be signaled by growth factors. In this communication, the authors show that the transforming growth factor α (TGF-α) gene is a direct target for p53-mediated transcriptional activation. In a stable cell line containing an inducible p53 construct, p53 induction leads to a threefold accumulation of the native TGF-α mRNA. In cotransfection assays using a TGF-α promoter **reporter** construct, the authors show that expression of wild-type but not mutant p53 increases transcriptional activity of the TGF-α promoter by .apprx.2.5-fold. In vitro, wild-type p53 binds to a consensus binding site found in the proximal portion of the promoter, and this sequence is necessary for the p53 transcriptional response. Furthermore, this element confers p53 induction to the otherwise nonresponsive **adenovirus** major late promoter. In addition to these results, the authors found that the TGF-α promoter contains a nonconsensus but functional TATA box-binding protein-binding site .apprx.30 bp upstream of the transcription start site. Although p53 can repress transcription from promoters containing a TATA box, the nonconsensus TGF-α TATA motif is resistant to this effect. On the basis of these results, the authors propose that p53 may play a dual role, which includes both the elimination of irreparably genetically damaged cells and the proliferative response necessary for their replacement, in the response to phys.-chemical damage.

L38 ANSWER 19 OF 20 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 95124309 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7823921
TITLE: Modulation of p53-mediated transcriptional repression and **apoptosis** by the **adenovirus** E1B 19K protein.
AUTHOR: Sabbatini P; Chiou S K; Rao L; White E
CORPORATE SOURCE: Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, New Jersey 08854.
CONTRACT NUMBER: CA53370 (NCI)
CA60088 (NCI)
SOURCE: Molecular and cellular biology, (1995 Feb) 15 (2) 1060-70.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950223
Last Updated on STN: 19980206
Entered Medline: 19950216

AB BRK cell lines that stably express **adenovirus** E1A and a murine temperature-sensitive p53 undergo **apoptosis** when p53 assumes the wild-type conformation. Expression of the E1B 19,000-molecular-weight

(19K) protein rescues cells from this p53-mediated **apoptosis** and diverts cells to a growth-arrested state. As p53 likely functions as a **tumor** suppressor by regulating transcription, the ability of the E1B 19K protein to regulate p53-mediated transactivation and transcriptional repression was investigated. In promoter-**reporter** assays the E1B 19K did not block p53-mediated transactivation but did alleviate p53-mediated transcriptional repression. E1B 19K expression permitted efficient transcriptional activation of the p21/WAF-1/cip-1 mRNA by p53, consistent with maintenance of the growth arrest function of p53. The E1B 19K protein is thereby unique among DNA virus-transforming proteins that target p53 for inactivation in that it selectively modulates the transcriptional properties of p53. The E1B 19K protein also rescued cells from **apoptosis** induced by inhibitors of transcription and protein synthesis. This suggests that cell death may result from the inhibition of expression of survival factors which function to maintain cell viability. p53 may induce **apoptosis** through generalized transcriptional repression. In turn, the E1B 19K protein may prevent p53-mediated **apoptosis** by alleviating p53-mediated transcriptional repression.

L38 ANSWER 20 OF 20 MEDLINE on STN
ACCESSION NUMBER: 95317378 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7796880
TITLE: A novel protein expressed in mammalian cells undergoing **apoptosis**.
AUTHOR: Grand R J; Milner A E; Mustoe T; Johnson G D; Owen D; Grant M L; Gregory C D
CORPORATE SOURCE: CRC Institute for Cancer Studies, University of Birmingham Medical School, Edgbaston, United Kingdom.
SOURCE: Experimental cell research, (1995 Jun) 218 (2) 439-51. Journal code: 0373226. ISSN: 0014-4827.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950817
Last Updated on STN: 19980206
Entered Medline: 19950728

AB Human and rodent cells undergoing **apoptosis** were observed to express high levels of a novel 45,000 M(r) protein. The protein, which we have termed **apoptosis** specific protein (ASP), was found in Burkitt lymphoma (BL) cells and in **adenovirus**-transformed human and rat embryo cells induced into **apoptosis** by a variety of stimuli, including serum deprivation, exposure to the Ca2+ ionophore, ionomycin, treatment with inhibitors of macromolecular synthesis (cycloheximide and actinomycin D), and cold shock. In BL cells treated with apoptotic stimuli, expression of the oncoprotein Bcl-2 was found to both protect from **apoptosis** and prevent expression of ASP. ASP was not detected either in viable cells or in cells dying passively by necrosis. Laser scanning confocal microscopy showed high levels of ASP in the cytoplasm of cells displaying the chromatin condensation and fragmentation patterns typical of **apoptosis**. Retention of ASP was observed even when DNA was no longer detectable, and two-color immunofluorescence staining indicated that the protein primarily colocalized with, but was clearly distinct from, non-muscle actin. These findings, together with the observation that biochemical extraction of ASP was only possible under conditions which caused solubilization of the cytoskeleton, leads us to conclude that ASP forms part of, or at least strongly associates with, a modified cytoskeleton unique to cells undergoing **apoptosis**. While elucidation of its function will require further work, ASP constitutes a powerful **marker** for the diagnosis and quantitation of **apoptosis** in vivo and in vitro.